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Senate House
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Bristol
BS8 1TH

Patents ADP number *(if you know it)*
If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

7972160001

4. Title of the invention
VACCINE

5. Full name of your agent *(if you have one)* — Haseltine Lake & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — Imperial House
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London WC2B 6UD

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United Kingdom	9809958.3	08/05/98

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Signature Date 28th May 1998

12. Name and daytime telephone number of person to contact in the United Kingdom — Dr. L.C. Sealy — [0117] 9260197

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VACCINE

This invention relates to an immunomodulator for use in a vaccine which is intended for use against a range of infectious agents. Further this invention relates to a vaccine composition comprising the immunomodulator, preferably in combination with antigen and a vaccination method using the vaccine composition.

Cholera toxin (Ctx) and its close relative E. coli heat-labile enterotoxin (Etx) are potent immunogens and mucosal adjuvants. However, their inherent toxicity makes them unsuitable for human use. For example, although Ctx is the most commonly used mucosal adjuvant in experimental animals, it is unsuitable for use in humans because of its potent diarrhoea-inducing properties. Attempts have been made to separate toxicity from adjuvant activity, for example by using components of Ctx and Etx as replacements for the holotoxins themselves. E. coli verotoxin (Vtx) is another known bacterial toxin.

Ctx and Etx are heterohexameric proteins composed of a an enzymatically active A subunit and a pentameric B subunit. CtxB and EtxB are known to bind GM1-ganglioside (GM1), a glycosphingolipid found ubiquitously on the surface of mammalian cells. Vtx binds to Gb3 which is a similar type of receptor to GM1.

In an attempt to circumvent the problem of toxicity for vaccine development, the adjuvant activity of the non-toxic B subunits has previously been investigated. However, many of the reports describe experiments in which a commercial preparation of CtxB or EtxB was used. These preparations are inevitably contaminated with a small but biologically significant amount of active toxin, so the adjuvant activity attributable to the B subunit is indistinguishable from the adjuvant activity of the whole toxin (Wu and

Russell (1993) Infection and Immunity 61: 314-322, US-5182109). Subsequent studies using recombinant CtxB (rCtxB) have suggested that CtxB is a poor mucosal adjuvant and only the addition of native holotoxin can provoke strong bystander responses (Tamura *et al* (1994) Vaccine 12: 419-426). Other studies have suggested that rCtxB lacks the ADP-ribosylating and the cAMP-stimulating activities of the holotoxin and that, as adjuvant mechanism is linked to these abilities, CtxB would be unsuitable for use as an adjuvant (Vajdy and Lycke (1992) Immunology 75: 488-492, Lycke *et al* (1992) Eur. J. Immunol. 22: 2277-2281, Douce *et al* (1997) Infection and Immunity 65: 2821-2828).

In another study, intranasal administration of ovalbumin using rCtxB as an adjuvant resulted in poor antibody responses. A non-toxic derivative of Ctx with a mutation in the A subunit also generated weak responses to bystander antigens, whereas the presence of an active A subunit dramatically enhanced adjuvant activity, suggesting that an active A subunit is essential (Douce *et al* (1997) as above).

It has also been shown that rCtxB and rEtxB can be used to promote tolerance to heterologous antigens (Sun *et al* (1994) Proc. Natl. Acad. Sci. 91: 4610-4614, Sun *et al* (1996) Proc. Natl. Acad. Sci. 93: 7196-7201, Bergerot *et al* (1997) Proc. Natl. Acad. Sci. 94: 4610-4614, Williams *et al* (1997) Proc. Natl. Acad. Sci. 94: 5290-5295), suggesting that these molecules would be unsuitable for use as adjuvants.

<u>The basis of the present invention</u>

In spite of the teaching in the art that CtxB and EtxB have poor adjuvanticity and can, in fact, act as tolerogens, the present inventors nevertheless investigated the use of rEtxB (thus containing no residual holotoxin or A subunit) in a intranasal

vaccine for HSV in a murine model and surprisingly found that it is able to stimulate protective immune responses to viral challenge. Specifically, the present inventors found that:

i) agents such as EtxB and CtxB stimulate high levels of local (mucosal) antibody production (although immunization using rEtxB stimulated lower levels of overall serum antibody production than Ctx/CtxB combined);

ii) the distribution of antibodies produced was skewed towards non-complement fixing antibodies, especially sIgA and IgG1;

iii) agents such as EtxB and CtxB also stimulated local and systemic T-cell proliferative responses;

iv) agents such as CtxB and EtxB tend to shift the immune response from a Th1-associated response to a Th2-associated response;

v) when agents such as CtxB and EtxB are used as immunomodulators some of the harmful effects of Th2-associated responses, such as the generation of IgE, are avoided;

vi) rEtxB is a more efficient immunomodulator than rCtxB;

vii) agents such as EtxB and CtxB are capable of altering the way in which an antigen presenting cell internalises and processes antigen, increasing antigen persistence;

viii) if an agent such as EtxB and CtxB is linked to an antigen, it is possible to alter the processing route of the antigen by altering the linkage to the immunomodulator; and

ix) VtxB exerts similar immunomodulatory effects on leukocyte populations in vitro to those exerted by EtxB and CtxB.

These important discoveries are the basis of the various aspects of the present invention and enabled

the inventors to predict that pure EtxB, CtxB and VtxB, as well as other agents capable of binding to or mimicking the effect of binding to GM1 or Gb3, will be useful as immunomodulators for use in vaccines in the prophylactic and therapeutic vaccination against HSV-1 infection, as well as other infections, the prevention or treatment of which would benefit from immunomodulation of the types listed above.

GM-1 and Gb3-associated signalling

Without wishing to be bound by theory, it is believed that GM1 or Gb3 binding may trigger intracellular signalling directly or indirectly. The present inventors have also found evidence which suggests that EtxB interacts with at least one other receptor which is involved in the GM1 associated intracellular signalling event. It may be that binding of EtxB (or CtxB) to GM1 facilitates binding to a protein, which protein triggers intracellular signalling. It is not known what specifically triggers the signalling event, it may be phosphorylation of GM1 or the protein. When EtxB/CtxB binds GM1 on the cell surface, bound GM1 is internalised in vesicles (Williams *et al* (1998) Infection and Immunity. In press). GM1 and other glycolipids (such as Gb3) are known to be preferentially located in "membrane rafts" in which key protein receptors are also found. It is therefore possible that internalisation of GM1 as a result of B-subunit binding causes cocapping of such proteins leading to their being triggered to mediate intracellular signalling events.

Definitions

An adjuvant is a substance which non-specifically enhances the immune response to an antigen, as distinct from a vaccine carrier, the purpose of which is to

target the antigen to a desired site. The term "immunomodulator" is used herein to indicate an agent which acts, like an adjuvant, to stimulate certain immune responses, but which also directs the immune response in a particular direction.

The term "coadministration" is used to mean that the site and time of administration of the antigen and immunomodulator are such that the necessary immune response is stimulated. Thus, while the antigen and the immunomodulator may be administered at the same moment in time and at the same site, there may be advantages in administering the antigen at a different time and/or at a different site from the immunomodulator.

The term "antigenic determinant" as used herein refers to a site on an antigen which is recognised by an antibody or T-cell receptor. Preferably it is a short peptide derived from or as part of a protein antigen, however the term is also intended to include glycopeptides and carbohydrate epitopes. The term also includes modified sequences of amino acids or carbohydrates which stimulate responses which recognise the whole organism.

The terms "CtxB", "EtxB" and "VtxB" as used herein include natural and recombinant forms of the molecule. The recombinant form is particularly preferred. They also include mutant molecules and other synthetic molecules (containing parts of CtxB, EtxB or VtxB) which retain the desirable immunological properties of CtxB, EtxB or VtxB. Agents other than EtxB and CtxB which retain GM1 binding activity, and agents other than VtxB which retain Gb3 binding activity include antibodies which bind GM1 or Gb3. Humanised monoclonal antibodies are especially preferred. In all aspects of the invention, the agent having GM1-binding activity or Gb3 binding activity may also be capable of cross-

linking GM1 or Gb3 receptors. EtxB is one such agent which is capable of cross-linking GM1 receptors by virtue of its pentameric form.

Stimulation of immune responses

EtxB, CtxB, VtxB and other agents capable of binding to or mimicking the effects of binding to GM1 or Gb3, are capable of acting as immunomodulators and stimulate specific immune responses to antigenic challenge.

According to a first aspect of the present invention, there is provided the use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

as an immunomodulator for a vaccine against infectious diseases.

According to a second aspect of the present invention, there is provided a vaccine composition for use against an infectious disease, which infectious disease is caused by an infectious agent, wherein the vaccine composition comprises an antigenic determinant and an immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

wherein said antigenic determinant is an antigenic determinant of said infectious agent.

The antigen and immunomodulator may be linked, for example covalently or genetically linked, to form a single effective agent, although in most applications of this aspect of the invention, separate administration (in which the antigen and immunomodulator are not so linked) is preferred because it enables separate administration of the different moieties.

According to a third aspect of the present invention, there is provided a kit for vaccination of a mammalian subject against an infectious disease, comprising:

a) one of the following agents:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding; and

b) an antigenic determinant which is an antigenic determinant of the infectious disease, for coadministration with the said vaccine immunomodulator.

The vaccine composition of the second aspect of the invention and the kit of the third aspect of the invention may be used in a prophylactic or therapeutic vaccination method, where a "prophylactic vaccine" is administered to naive individuals to prevent disease development, and a "therapeutic vaccine" is administered to individuals with an existing infection to reduce or minimise the infection or to abrogate the immunopathological consequences of the disease.

According to a fourth aspect of the present invention there is provided a method of preventing or treating a disease in a host, which method comprises the step of inoculating said host with a vaccine

comprising at least one antigenic determinant and an immunomodulator, where the immunomodulator is:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding.

The vaccine may be administered by a number of different routes such as intranasal, oral, intra-vaginal, urethral or ocular administration. Intranasal immunisation is preferred.

The antigenic determinant and immunomodulator may be administered to the subject as a single dose or in multiple doses.

Stimulation of mucosal immune responses

EtxB, CtxB, VtxB and other agents capable of binding to or mimicking the effects of binding to GM1 or Gb3, are capable of specifically upregulating mucosal antibody production.

The vaccine immunomodulator of the first aspect of the invention, the vaccine composition of the second aspect of the invention and the kit of the third aspect of the invention are particularly effective against diseases where protection from infection or treatment is effected *in vivo* by a mucosal immune response. For example, against diseases in which, during infection, the infectious agent binds to, colonises or gains access across the mucosa. Examples of such diseases include, diseases caused by viruses (HIV, HSV, EBV, CMV, influenza, measles, mumps, rotavirus etc), diseases caused by bacteria (E. coli, Salmonella, Shigella, Chlamydia, N. gonnorhoea, T. pallidium, Streptococcus species including those which cause

dental caries), and diseases caused by parasites.

In a preferred embodiment of the second aspect of the present invention there is provided a vaccine against HSV-1 infection comprising at least one HSV-1 antigenic determinant and an immunomodulator, where the immunomodulator is:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or G3b binding.

Preferably the immunomodulator is EtxB.

In a preferred embodiment of the third aspect of the present invention there is provided a kit for vaccination of a mammalian subject against an HSV-1, comprising:

a) a vaccine immunomodulator which is:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or G3b binding; and

b) at least one HSV-1 antigenic determinant, for coadministration with the said vaccine immunomodulator.

According to a fifth aspect of the invention there is provided the use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular

signalling events mediated by GM1-binding or Gb3 binding

to upregulate the production of antibodies at mucosal surfaces. The production of non-complement-fixing serum antibodies may also be upregulated. Preferably, sIgA is produced in accordance with the fifth aspect of the invention.

In this fifth aspect of the present invention, the agent may be used in conjunction with one or more antigenic determinant(s).

Downregulating the pathological components of immune responses

The inventors also found that when pure EtxB was used as an immunomodulator in the described way, the harmful effects of Th2 associated responses, such as the generation of high levels of potentially pathological IgE, were avoided. Despite this, the immune response triggered by the use of EtxB (or CtxB or VtxB) as an immunomodulator appears to favour the induction of Th2-associated cytokines. In other words EtxB (or CtxB) induces a shift from a Th1- to a Th2-type response. This has enabled the inventors to predict that pure EtxB, CtxB or VtxB, as well as other agents capable of binding to or mimicking the effect of binding to GM1 or Gb3, will be capable of down regulating pathological components of the immune response associated with both Th1 and Th2 activation.

According to a sixth aspect of the present invention, there is provided the use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3

binding;

to downregulate the pathological components of Th2-associated immune responses. The pathological components of Th1-associated immune responses may also be downregulated.

It is known that EtxB and CtxB bind to GM1 and induce differential effects on lymphocyte populations, including a specific depletion of CD8+ T cells and an associated activation of B cells (WO 97/02045). Hence, EtxB and CtxB are thought to alter the balance of the immune response such that inflammatory Th1 associated reactions are down-regulated while Th2 associated responses are upregulated. Th1 responses include the secretion of γIFN by activated T-cells leading to macrophage activation and delayed type hypersensitivity reactions. Such responses may be an important cause of pathology during infections with a number of pathogens. Th2 responses include the activation of T-cells to produce cytokines such as IL-4, IL-5, IL-10, and are known to promote the secretion of high levels of antibody, especially IgA.

It has now surprisingly been found that when EtxB is used as an immunomodulator in the described way, the harmful effects of Th2 associated responses, such as the generation of high levels of potentially pathological IgE, are avoided. Therefore, EtxB and CtxB are capable of down regulating pathological components of the immune response associated both with Th1 and Th2 activation. Such responses are modulated in favour of the production of high levels of non-complement fixing serum antibodies and secretory IgA production at the mucosal surfaces.

The use of an agent in accordance with the sixth aspect of the invention is particularly useful for therapeutic vaccination in diseases in which immunopathological mechanisms are involved. Examples

of such diseases are HSV-1, HSV-2, TB and HIV.

The first and sixth aspects of the invention can be combined. In other words, agents such as EtxB can be used simultaneously as an immunomodulator and a therapeutic agent. For example in diseases where immunopathological mechanisms are involved, the use of a vaccine incorporating agents such as EtxB or CtxB may act not only to limit infection, but also to abrogate the pathological disease processes. The immunomodulating agent is thus acting both prophylactically and therapeutically. Examples of infections where vaccination in this way is therefore likely to be of particular value include those caused by the herpes virus family, measles, gastrointestinal and respiratory tract pathogens.

Immunomodulation of the antigen processing pathway

a) prolonging presentation

The present inventors have also found that when EtxB (or CtxB or VtxB) is used as an immunomodulator, the antigen internalisation and processing pathway is altered. The presence of the B subunit causes prolonged presentation, possibly by altering antigen trafficking inside the antigen presenting cell such that antigen degradation is delayed and therefore maintained over longer periods. This feature of B-subunit associated antigen presentation means that vaccines incorporating an agent in accordance with the present invention will have increased antigen persistence and lead to sustained immunological memory.

According to a seventh aspect of the present invention, there is provided the use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

as an immunomodulator in a vaccine, to prolong antigen presentation and give sustained immunological memory in a mammalian subject.

According to an eighth aspect of the present invention, there is provided a vaccine composition for use against an infectious disease, comprising an antigenic determinant and a immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

wherein said antigenic determinant is an antigenic determinant of said infectious disease and wherein the immunomodulator prolongs presentation of the antigenic determinant and gives sustained immunological memory.

b) intracellular targeting of the antigen to a MHC-I or MHC-II associated pathway

As aforementioned, the antigen and immunomodulator in a therapeutic or prophylactic vaccine may be linked, for example covalently or genetically linked, to form a single effective agent. The present inventors have found that is possible to direct the antigen to different compartments of the cell and hence to different antigen presentation pathways by altering the linkage of the antigen to the immunomodulator.

By linking the antigen or antigenic determinant to the immunomodulator in a certain way, it is possible to facilitate translocation of the antigen across the

endosomal membrane into the cytosol. The present inventors predict that this would enhance loading of antigenic peptides on to MHC class I molecules. The use of an antigen-immunomodulator conjugate can therefore be used to specifically enhance the activation of cytotoxic T cells (CTL). Induction of CTL is beneficial for the prevention and treatment of many diseases especially those caused by viruses, intracellular bacteria and parasites.

The linkage of the antigen-immunomodulator conjugate can also be chosen so that the antigen is delivered into the nucleus.

According to a ninth aspect of the present invention there is provided a conjugate comprising an antigen or antigenic determinant and an immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding.

According to a tenth aspect of the present invention there is provided a vaccine composition for use against an infectious disease, which infectious disease is caused by an infectious agent, which vaccine composition comprises a conjugate of an antigen or antigenic determinant and an immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or G3b binding;

wherein said antigen or antigenic determinant is

an antigen or antigenic determinant of said infectious agent.

The antigen or antigenic determinant may be linked to the immunomodulator by a variety of methods including genetic linkage or chemical conjugation. In a first preferred embodiment the conjugate is a fusion protein made by genetic linkage of the antigen or antigenic determinant to the immunomodulator. Preferably the antigen or antigenic determinant is genetically linked to the C-terminus of the immunomodulator. In a second preferred embodiment the antigen or antigenic determinant is chemically conjugated to the immunomodulator. Preferably the antigen or antigenic determinant is conjugated to the immunomodulator using heterobifunctional cross-linking reagents. More preferably the cross-linking agent is N-γ(-maleimido-butyroxyl)-succinimide ester (GMBS) or N-succinimidyl-(3-pyridyl-dithio)-propionate (SPDP).

The vaccine composition may be administered by a number of different routes such as intranasal, oral, intra-vaginal, urethral or ocular administration. Intranasal immunisation is preferred.

According to an eleventh aspect of the present invention there is provided the use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding;

in a conjugate with antigen or antigenic determinant to target the delivery or said antigen or antigenic determinant to the cytosol or nucleus of an antigen presenting cell.

According to a twelfth aspect of the present invention there is provided the use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding;

in a conjugate with antigen or antigenic determinant to upregulate the presentation of said antigenic determinant, or an antigenic determinant derived from said antigen, by MHC class I molecules.

Preferably the use of the conjugate of the twelfth aspect of the invention is used is combination with the use of the agent in accordance with the fifth aspect of the invention to stimulate strong CTL responses and to upregulate mucosal antibody production. This activity would be particularly useful in the prevention and treatment of viral infections, for example influenza.

<u>EtxB is the preferred immunomodulator</u>

It has previously been thought that EtxB and CtxB have similar properties. However, the present inventors have found that rEtxB is a more potent and efficient immunomodulator than rCtxB. Hence the preferred immunomodulator is EtxB, or agents which mimic the effects of EtxB.

The invention will now be illustrated by reference to the accompanying drawings and the following examples.

The examples refer to the figures in which:

Figure 1: shows the stimulation of total Ig and IgA in the serum (MS) and IgA in the eye washings (EW) in mice immunised with HSV-1 glycoproteins/rEtxB.

Figure 2: shows T cell proliferation of (mesenteric lymph node) MLN or (cervical lymph node) CLN lymphocytes in mice immunised with HSV-1/rEtxB.

Figure 3: shows T cell proliferation of cells from MLN and CLN of mice immunised intranasally with HSV-1 Gp in the presence of 1-20μg EtxB.

Figure 4: shows the level of anti-HSV-1 serum Ig in mice following administration of HSV-1 glycoproteins three times at 10 day intervals with variable amounts of rEtxB or rCtxB as adjuvant.

Figure 5: shows the reduction in virus shedding, clinical disease and latency in mice immunised with HSV-1/rEtxB.

Figure 6: shows the Ig isotype distribution in MS following infection with HSV-1 or immunisation with HSV-1 Gp in the presence of EtxB or CtxB as immunomodulator.

Figure 7: shows the distribution of Ig subclasses following intranasal administration of HSV-1 Gp with either rEtxB or rCtxB as immunomodulator.

Figure 8: shows the immunogenic effect of different amounts of rEtxB or rCtxB on the level of HSV-1 specific IgA in eye washings following administration with HSV-1 glycoproteins.

Example 1: rEtxB can be used in conjunction with HSV-1 Gp for immunisation.

Mice were immunised intranasally three times with 10γg HSV-1 plycoproteins (Gp) with either 10 or 20γg rEtxB. Controls were either unmanipulated or given a mock preparation of viral glycoprotein (mock) derived from HIV-uninfected tissue culture cells. Antibody levels are expressed as a percentage of post-infection levels. The production of total Ig and IgA in the serum and IgA in eye washings was stimulated by HSV-1 glycoproteins/rEtxB (Figure 1). The present inventors have also shown that doses of rEtxB as low as 0.1γg are also effective at stimulating such responses.

Also, T-lymphocytes from immunised mice from the

cervical lymph node (which is local to the vaccination site) and from the mesenteric lymph node (which is distant to the vaccination site) were shown to proliferate when cultured in vitro with HSV-1, but not when cultured in vitro with mock HSV-1 Gp or without antigen (Figure 2).

The proliferation in response to HSV-1 Gp of T lymphocytes from MLN and CLN of mice immunised with HSV-1 Gp and varying amounts of EtxB is shown in Figure 3.

The production of Anti-HSV-1 serum Ig in mice following administration of HSV-1 glycoproteins at three day intervals with varying amounts of EtxB (or CtxB) is shown in Figure 4.

Finally, mice immunised with HSV-1 and rEtxB were shown to have a decrease in virus shedding following corneal scarification with HSV-1 (Figure 5a), and a decrease in local spreading (oedema and lid disease), spreading to the trigeminal ganglion (zosteriform infection), spreading to the central nervous system (encephalitis) and latency compared to control mice (Figure 5b).

Example 2: rCtxB and rEtxB act as immunomodulators.

When EtxB is used as an immunomodulator, the Ig isotype distribution is skewed (Figure 6). The distribution of Ig subclasses is different depending on whether rCtxB or rEtxB is used as an immunomodulator (Figure 7).

Example 3: rEtxB is a more efficient immunomodulator than rCtxB.

The levels of HSV-specific IgA (Figure 8) and is greater following stimulation with rEtxB/HSV-1 Gp that rCtxB/HSV-1 Gp.

CLAIMS

1. The use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

as an immunomodulator for a vaccine against infectious diseases.

2. A vaccine composition for use against an infectious disease, which infectious disease is caused by an infectious agent, wherein the vaccine composition comprises an antigenic determinant and an immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

wherein said antigenic determinant is an antigenic determinant of said infectious agent.

3. A vaccine composition according to claim 2 in which the infectious disease is HSV-1 infection and wherein the antigenic determinant is an antigenic determinant of HSV-1.

4. A vaccine composition according to claim 2 or 3 in which the immunomodulator is EtxB free from whole toxin.

5. A kit for vaccination of a mammalian subject against an infectious disease, which kit comprises:

a) one of the following agents:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding; and

b) an antigenic determinant which is an antigenic determinant of the infectious disease, for coadministration with the said vaccine immunomodulator.

6. A method of preventing or treating a disease in a host, which method comprises the step of inoculating said host with a vaccine comprising at least one antigenic determinant and an immunomodulator, where the immunomodulator is:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding.

7. The use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding

to upregulate the production of antibodies at mucosal surfaces.

8. The use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

as an immunomodulator in a vaccine, to prolong antigen presentation and give sustained immunological memory in a mammalian subject.

9. A vaccine composition for use against an infectious disease, which infectious disease is caused by an infectious agent, which vaccine comprises an antigenic determinant and a immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

wherein said antigenic determinant is an antigenic determinant of said infectious agent and wherein the immunomodulator prolongs presentation of the antigenic determinant and gives sustained immunological memory.

10. The use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding;

in a conjugate with antigen or antigenic determinant to target the delivery or said antigen or antigenic determinant to the cytosol or nucleus of an antigen presenting cell.

11. The use of:

(i) EtxB, CtxB or VtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having

GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding;

in a conjugate with antigen or antigenic determinant to upregulate the presentation of said antigenic determinant, or an antigenic determinant derived from said antigen, by MHC class I molecules.

Level of Ig or IgA in MS or IgA in EW compared with control mice following immunisation with HSV-1 or mock Gp preparations with different amounts of rEtxB

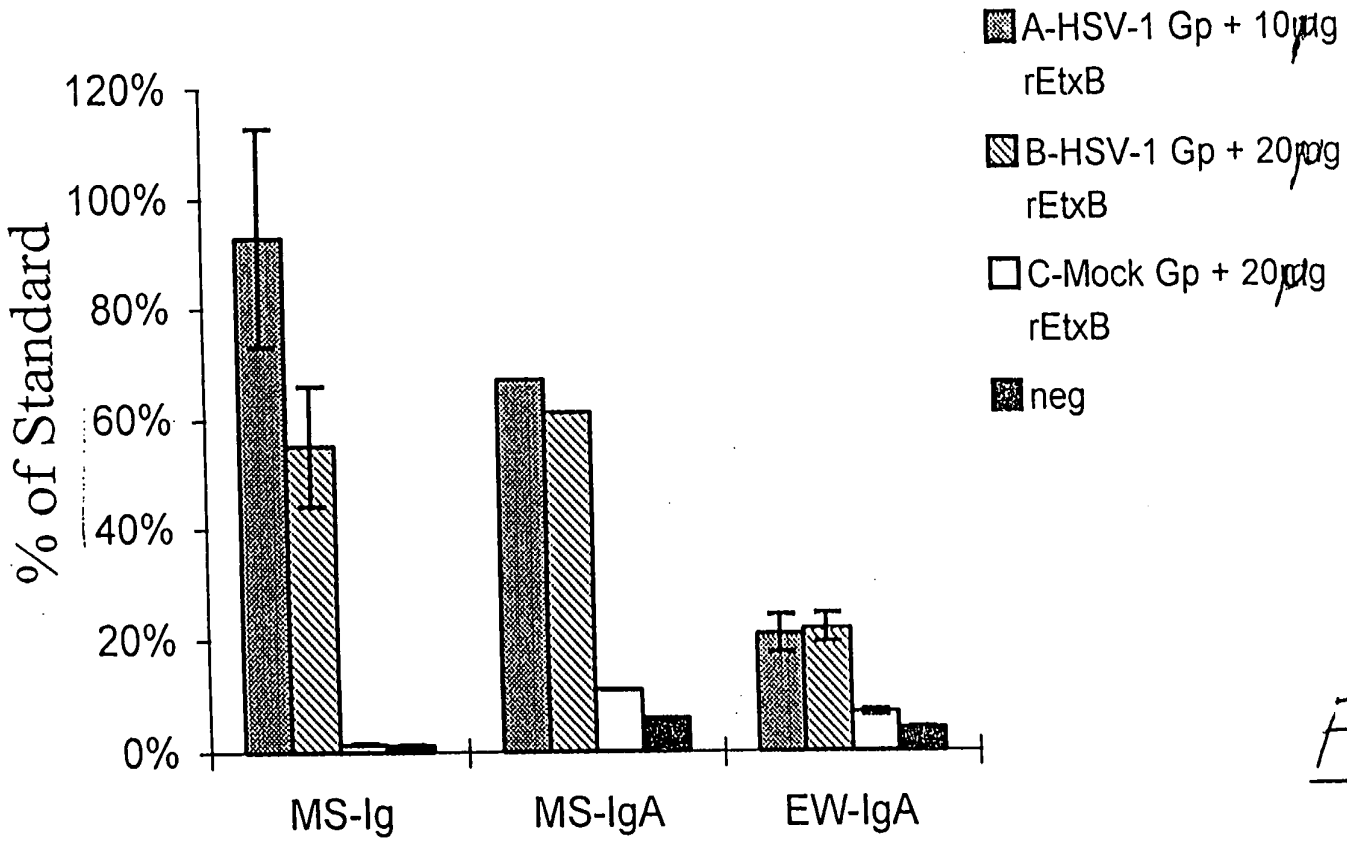


FIGURE 1

T cell proliferation of MLN or CLN lymphocytes from mice given HSV-1 GP with 10μg (A), 20μg (B) rEtxB or mock Gp with 20μg rEtxB (C) by the i.n. route cultured *in vitro* with different antigens

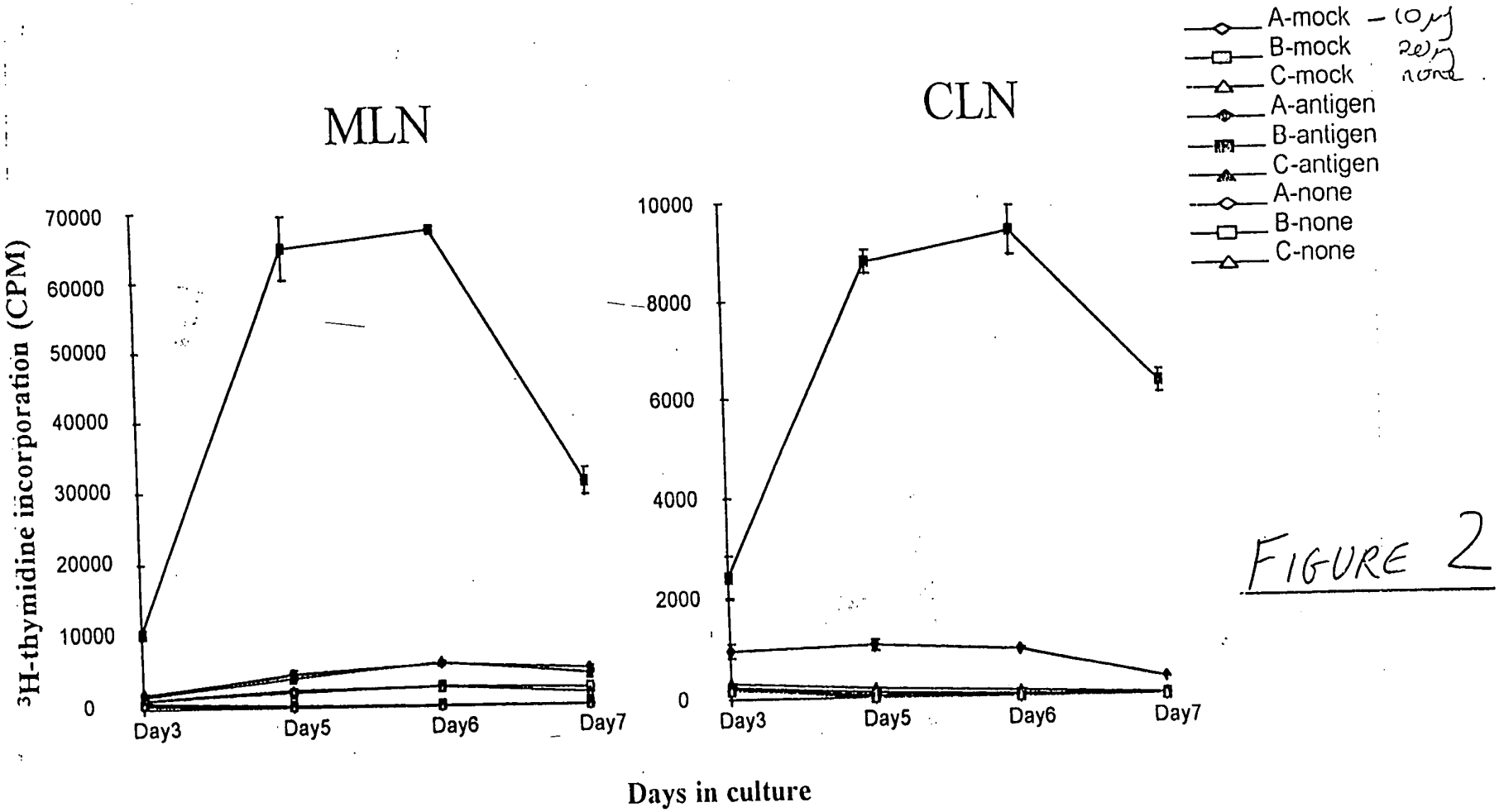


FIGURE 2

2/6

Tcell proliferation of cells from MLN and CLN of mice immunised i.n. with HSV-1 Gp in the presence of 1-20μg EtxB as adjuvant

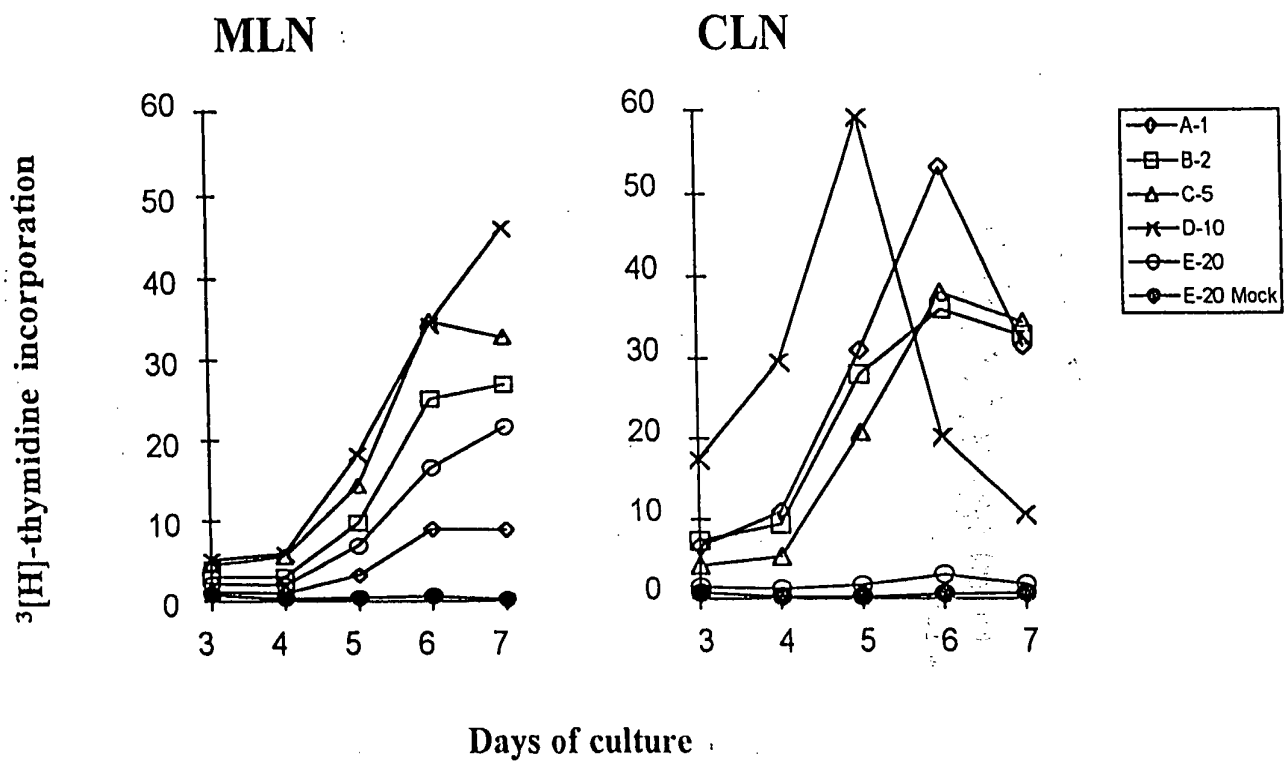


FIGURE 3

Anti-HSV-1 serum Ig in mice following administration of HSV-1 glycoproteins three times at 10 day intervals with variable amounts of rEtxB or rCTB as adjuvant

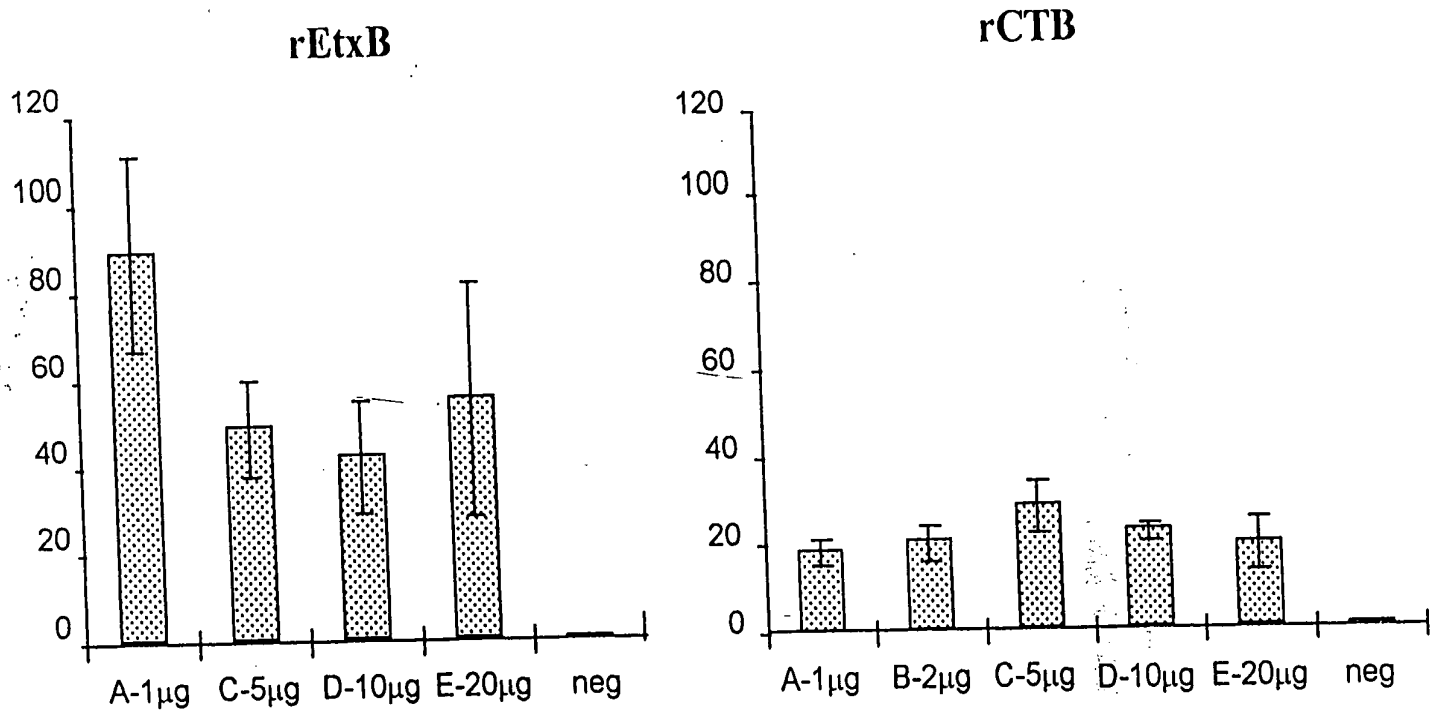


FIGURE 4

Figure 5a. Incidence of virus shedding from the eye following corneal scarification of mice with HSV-1 (SC16)

Day post infection	10μg rEtxB + HSV-1 gp (%)[1]	20μg rEtxB + HSV-1 gp (%)	20μg rEtxB + mock gp[2] (%)
1	0	30	60
2	60	80	95
3	60	80	95
6	10	0	70
7	10	0	70
8	0	0	10
9	0	0	0

[1] Percentage of animals from which wash fluid from the eye secretions revealed the presence of live viral particles in a plaque assay.
[2] Mock infected animals were given an inoculum of glycoproteins prepared from uninfected tissue culture cells.

Figure 5b. Clinical disease following corneal scarification of mice with HSV-1 (SC16)

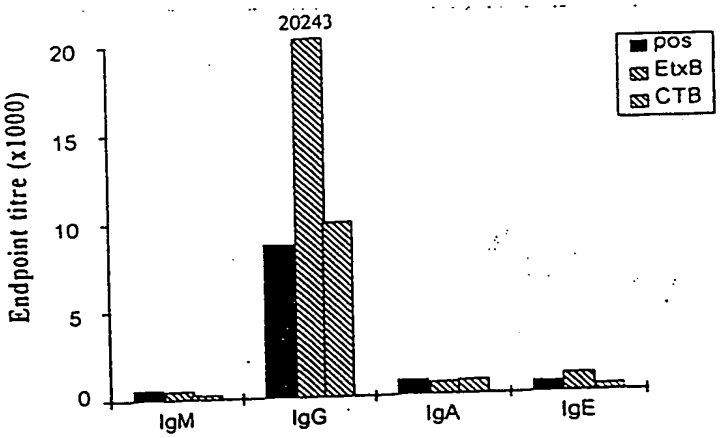
	Corneal ulcers[2]	Oedema	Lid disease	Zosteriform infection	Encephalitis	Latency[1] TG1	TG2	TG3
10μg rEtxB + HSV-1 gp	80%	0%	0%	0%	0%	22%	11%	0%
20μg rEtxB + HSV-1 gp	70%	0%	0%	0%	0%	80%	10%	0%
20μg rEtxB + mock gp	80%	45%	55%	40%	40%	83%	30%	16%

[1] Latency was determined by extraction of the trigeminal ganglion (TG) from surviving mice 2 months after infection and coculturing with Vero cells. Figures given are for each of the lobes of the TG (TG1, TG2 and TG3).
[2] Figures are percentage of animals showing signs of the described symptoms at any point during acute infection. Each mouse was examined on a daily basis during the first 11 days of infection.

FIGURE 5

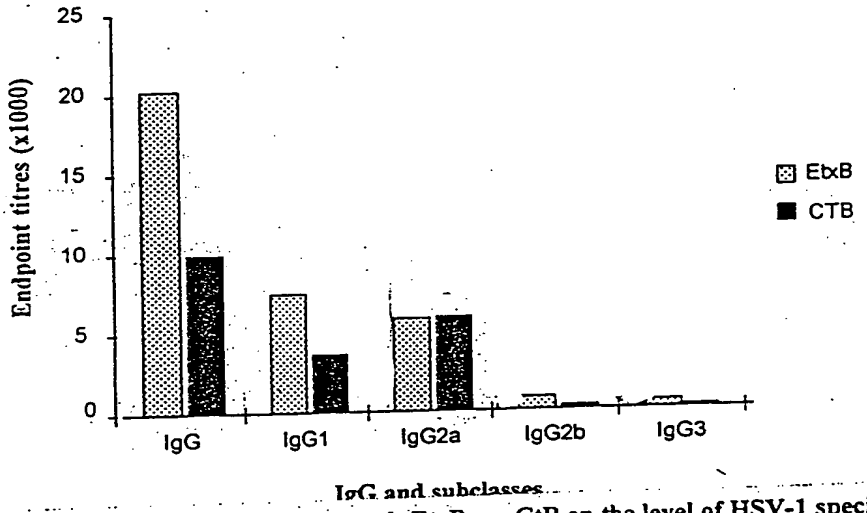
Ig Isotype distribution in MS from mice following infection (pos) or immunisation with H[redacted] Gp in the presence of EtxB or CTB a[redacted]juvant

FIGURE 6



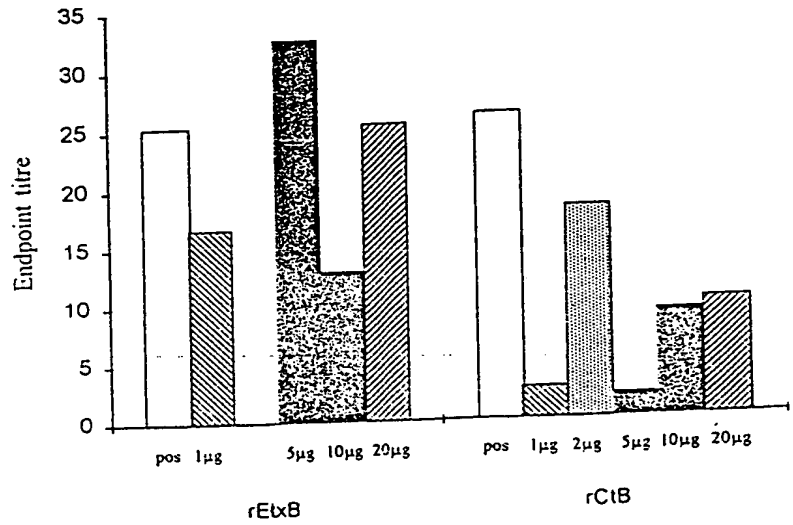
Distribution of subclasses following administration of HSV-1 Gp i.n. with either rEtxB or rCTB as adjuvant

FIGURE 7



Adjuvant effect of different amounts of rEtxB or rCtB on the level of HSV-1 specific IgA in eye washings following administration with HSV-1 glycoproteins

FIGURE 8



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Form 23/77 : 10/5/99

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